FIELD TRIALS OF ATTENUATED *SALMONELLA TYPHI* LIVE ORAL VACCINE TY21A IN LIQUID AND ENTERIC-COATED CAPSULE FORMULATIONS IN SANTIAGO, CHILE

INTERIM REPORT

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In a randomized, double-blind, controlled field trial in Santiago, Chile, 81,621 school-children, 5-19 years of age, received three doses, within one week, of attenuated Salmonella typhi oral vaccine Ty21a in enteric-coated capsules or in a new liquid suspension, or placebo. OVer 36 months of surveillance, the liquid formulation (76.9% vaccine efficacy) was markedly superior to enteric-coated capsules (33.2% vaccine efficacy) (p=0.0000077). The liquid formulation protected young children (5-9 year olds) (vaccine efficacy 82.3%) as well as older children (≥ 10 years) (69.3% vaccine efficacy), while the capsules significantly protected only older children. The liquid suspension was practical to prepare by mixing lyophilized vaccine with buffer in water and was easily administered, even to the youngest children. We conclude that the liquid formulation of Ty21a is preferable to enteric-coated capsules.

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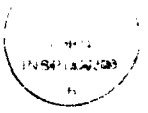
FOREWORD

The investigator(s) have abided by the National Institutes of Health Guidelines for Research Involving Recombinant DNA Molecules (April 1982) and the Administrative Practices Supplements.



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INTRODUCTION

It is estimated that circa 33 million cases of typhoid fever still occur annually worldwide (1). Populations at particular risk include schoolage children in many less-developed countries (2-5), travelers from industrialized countries who visit such regions (6) and clinical microbiology technicians (7). In December 1989, the Food and Drug Administration licensed a new live oral typhoid vaccine, strain Ty21a. This Vi-negative, *galE* mutant attenuated strain of *Salmonella typhi* (8), used as a live oral vaccine, represents an advance in immunoprophylaxis against typhoid fever because it significantly protects without causing adverse reactions (9-14). Field trials of Ty21a have shown that the formulation of vaccine used and the number of doses administered markedly affect the level of efficacy that can be achieved (11-14).

In the first controlled field trial of Ty21a, carried out in schoolchildren in Alexandria, Egypt, three doses (10^9 viable vaccine organisms per dose) given on an every other day schedule provided 96% protection against culture-confirmed typhoid fever during three years of surveillance (10,15). In Alexandria, Ty21a was given as a liquid suspension prepared by reconstituting, in the field, a one-dose vial of lyophilized vaccine with a vial of diluent (10,15); the vaccine suspension was administered to each child several minutes after the child ingested 1.0 gm of sodium bicarbonate to neutralize gastric acid.

Because the formulation used in Alexandria was deemed impractical to mass produce, two subsequent formulations were manufactured and evaluated in randomized, controlled trials in Santiago, Chile (11). One formulation consisted of lyophilized vaccine in gelatin capsules accompanied by two additional gelatin capsules each containing 0.5 gram of sodium bicarbonate. The second formulation consisted of enteric-coated capsules that do not require accompanying buffer as the capsules open only in an environment with a pH $\geq$ 6.0. The

enteric-coated capsules were significantly superior to the gelatin capsule/sodium bicarbonate formulation (11). Three doses of Ty21a in enteric-coated capsules given within one week provided 67% protection during the first three years of follow-up and 66% protection for five years in a field trial in Area Occidente, Santiago (11,15). A subsequent field trial carried out in two other administrative areas of Santiago (Sur and Central) showed that, in a direct comparison, an immunization schedule of four doses of Ty21a given within a period of eight days was significantly more protective than three doses (12). Based on these data, the FDA licensed the enteric-coated capsule formulation of Ty21a with a recommended immunization schedule of four doses to be given on days 1, 3, 5, and 7.

While the enteric-coated capsule formulation of Ty21a proved practical and moderately efficacious, epidemiologists and public health officials continued to be intrigued by the high three year efficacy (96%) recorded in the Alexandria trial (10) versus the Area Occidente, Santiago field trial (67%) (11). Possible explanations that have been considered for the higher efficacy in Alexandria include (14): 1) differences in the immune response to Ty21a based on genetic variations in the two human populations; 2) differences in the antigenic make-up of endemic *S. typhi* strains in Alexandria versus Santiago; 3) differences in the modes of transmission of *S. typhi* resulting in generally higher inocula ingested in Santiago (mainly food-borne) than in Alexandria (mainly water-borne); 4) statistical considerations; 5) an inherent superiority of a liquid suspension over an enteric-coated capsule formulation.

Formulation was the one variable that was amenable to evaluation in a prospective field trial and the development of a new liquid formulation made a comparative trial feasible. Therefore, we initiated a randomized, double-blind, controlled field trial in two administrative areas of Santiago, Chile, in order to directly compare the efficacy of three doses of Ty21a in enteric-coated capsules versus a liquid suspension.

MATERIALS AND METHODS

Ethical Clearances and Informed Consent Procedures

Advantages of carrying out field trials of typhoid vaccines in Santiago, Chile include a high incidence of the disease in schoolchildren (3,11,12), an excellent health care infrastructure (11,12,16) and prior experience in organizing large-scale typhoid vaccine field trials (11,12). The trial design and consent procedures were approved by ethical review committees of the University of Maryland School of Medicine, the World Health Organization, Geneva and the U.S. Department of Defense, Washington, D.C.

The Ministries of Health and of Education collaborated to ensure that parents and guardians were informed of the trial by distribution of an informational brochure that was prepared by the Health Education Unit of the Ministry of Health. Permission to enroll each child was then requested by means of consent forms and the parental response was recorded.

Rationale for Inclusion of a Control Group

The fact that in earlier trials Ty21a had been shown to confer significant protection against typhoid fever (11,14,17) created an ethical dilemma with respect to having a placebo control group. Nevertheless, the need to measure the absolute efficacy for each formulation, in addition to the relative efficacy, dictated inclusion of a control group. This dilemma was resolved in the following way: 1) The size of the control group was reduced to the minimum number required to give statistical significance. Based on estimates of vaccine efficacy for each formulation and predictions of incidence rates in the control and vaccine groups (*vide infra*), this proved to be one placebo child for every seven vaccine recipients. 2) At the time of initiating this trial, Ty21a had not been introduced as a routine immunization for schoolchildren in Santiago or elsewhere in Chile. Thus children in the control group were not

being deprived of a routine vaccine that they would otherwise receive. 3) Instead of an inactive placebo, children in the control group received viable *Lactobacillus acidophilus* because some experimental data suggest that *L. acidophilus* may provide short-term protection against diarrhea due to enterotoxigenic *Escherichia coli* (18,19), another pathogen that is endemic in Santiago (20-22). Consequently the control children may have derived some short-term benefit against *E. coli* diarrhea by participating in the vaccine trial, although their risk of typhoid fever was not diminished.

Formulations of Vaccine and Placebo and the Method of Vaccination

Hydroxyprophylmethylcellulosephthalate was the enteric-coating used to make the capsules acid-resistant. Such capsules, each containing 1-3 x 10^9 viable vaccine (or *Lactobacillus*) organisms, resist gastric acid (pH 1.5) for at least two hours but dissolve within 10 minutes in artificial intestinal fluid of Ph $\geq$6.0 (11).

The liquid formulation of vaccine (or of the *Lactobacillus* control preparation) consisted of two aluminum foil packets, one containing lyophilized vaccine (or *Lactobacillus*) (1-3 x 10^9 viable organisms) and 25 mg aspartame and the other containing a powdered buffer (2.65 g $NaHCO_3$ and 1.65 g ascorbic acid). To prepare a dose, contents of a packet of lyophilized vaccine (or *Lactobacillus*) and a packet of buffer were mixed together in a cup with 100 ml of water and the buffered suspension was ingested by the child.

Calculation of Sample Sizes

During the two years prior to the trial, the incidence of typhoid fever in children 5-19 years of age in Area Sur Oriente was 103 cases per 10^5. The maximum population of schoolchildren theoretically available for this field trial was 173,589, including all 137,767 5-19 year olds in Area Sur Oriente and 36,822 5-9 year olds in Area Norte. Based on experiences in our three previous large-scale field trials in Santiago, a parental consent rate as low as 60%

or as high as 96% could be expected. Thus, depending on the parental consent rate, as few as 104,153 or as many as 166,822 schoolchildren might actually be available for randomization.

Accordingly, two sets of sample size estimates were prepared, one extremely conservative and the other closer to expectation based on incidence rates from prior surveillance and previous measurements of efficacy of Ty21a. Assumptions in the calculation of sample sizes included three years of surveillance, a statistical power of 80%, $p < 0.025$ for detecting absolute efficacy versus the control group (single tail hypothesis), $p < 0.05$ (2-tails) for detecting relative efficacy between the two formulations of vaccine with Ty21a in enteric-coated capsules estimated to provide 60% efficacy for at least three years.

Based on the above assumptions, at a conservative estimated incidence of 70 cases per 10^5 children in the *Lactobacillus* control group, inclusion of 13,800 children in the *Lactobacillus* control group and 54,000 in each of the two vaccine groups would allow detection of a vaccine efficacy of at least 60% and a significant difference between the two vaccine formulations if the liquid formulation were 20% more efficacious than the capsules. As few as 31,085 children per vaccine group would suffice to detect a significant difference if the level of efficacy conferred by the formulations varied by at least 25%.

Using a less conservative estimated incidence of 90 cases of typhoid per 10^5 in the *Lactobacillus* control group, inclusion of 10,743 children in the *Lactobacillus* control group and 42,236 children in each of the two vaccine groups would allow detection of vaccine efficacy of at least 60% and would also permit detection of a significant difference between the formulations if the liquid formulation were 20% more efficacious than the capsules. As few as 24,174 children in each vaccine group would suffice to observe a significant difference if the levels of protection differed by at least 25%.

Randomization

In Santiago, peak transmission of typhoid fever and the vast majority of cases occur during the summer season from mid-December to mid-March when schools are not in session (4,11,12). Consequently, vaccination was carried out in September and October, several months before onset of the typhoid season. In order to simplify the logistics of the school-based vaccination, randomization was done by classroom so that all children of consenting parents within a class received the same vaccine regimen, i.e. either vaccine or placebo in enteric-coated or liquid formulation.

Assuring Blindness of the Study

The packets and the enteric-coated capsules containing control preparations (1-3 x 10^9 viable *L. acidophilus* organisms) appeared identical to those containing attenuated *S. typhi* vaccine. The study was double-blind with neither the trial organizers, vaccinators, the children or their parents, or health care providers knowing the identity of the preparation (i.e. vaccine or placebo) given to any child. One-eighth of the participating children received the *Lactobacillus* control preparation. Since the number of control children was much smaller than the number who were to receive vaccine, in order to maintain double blindness, each formulation was evenly divided into eight separate letter codes; one letter represented the control preparation and the other seven signified vaccine. The code was kept by the Diarrhoeal Diseases Control Programme of WHO and was not broken until 36 months of surveillance had been completed. Results were analyzed by chi-square for statistical significance.

Epidemiological Surveillance Methods

The vaccination was carried out by trained health workers in the classrooms during September and October, 1986 after which computerized data files were generated from the

completed class lists. Surveillance began in November, 1986. Approximately 90% of health care visits in Area Sur Oriente and Area Norte occur in facilities of the National Health Service where intensive surveillance could be maintained; the remaining visits are to private practitioners. Physicians and nurses were kept aware of the importance of obtaining cultures from suspect cases of typhoid fever by means of clinical conferences, letters from the Ministry and weekly visits by surveillance nurses from the Typhoid Fever Control Program. Only confirmed cases, i.e. those from which *S. typhi* was isolated from blood, bone marrow or bile-stained duodenal fluid cultures (11,12,23), were used to compute vaccine efficacy. Considerable resources were therefore expended to ensure bacteriological confirmation of suspect cases. From hospitalized children three 4 ml blood cultures were obtained, sometimes accompanied by a bone marrow culture (23). Two 6 ml blood cultures were obtained 30 minutes apart from outpatients presenting to health centers with suspected typhoid fever (11). Suspect colonies were examined by standard biochemical and serological techniques (23).

RESULTS

Parents of 64% of the eligible schoolchildren gave consent for their child to participate; 95,910 of these children received at least one dose of vaccine or placebo (i.e. the *Lactobacillus* control preparation), including 64,413 in Area Sur Oriente and 31,497 in Area Norte. The children in Area Sur Oriente ranged in age from 5-19 years. In contrast, since a previous field trial had been conducted in Area Norte in 1982 (14,17), participation in Area Norte was limited to children who entered the schools after the 1982 vaccine trial, i.e. 5-9 year olds. A total of 81,621 children received the full three scheduled doses of vaccine or placebo. During the vaccination period there was no increased absenteeism or any significant increase

in febrile or gastrointestinal illnesses and no cases of typhoid fever were recorded among the participating children.

Results of 36 months of surveillance in this field trial are summarized in Table 1 where the incidence data are primarily presented as cases of typhoid per 10^5 schoolchildren. While both formulations of Ty21a provided protection against confirmed typhoid fever, the overall level of protection conferred by the liquid formulation (76.9% efficacy) was significantly superior to that conferred by the enteric-coated capsule formulation (33.2% efficacy) ($p=0.0000077$).

Since the risk of developing typhoid fever is essentially independent of school attendance in Santiago, randomization in this trial was carried out by class to simplify the logistics of vaccination. It was thus critical to carefully analyze the data and verify that no clustering of cases had occurred. Cases of typhoid fever were observed in 374 of the 5423 classes in Sur Oriente and Norte, including cases in non-participants. Only 18 of these 374 classes (4.8%) had more than one case of typhoid fever (17 classes had two and one class had three); in all but three instances, these cases were temporally unrelated, occurring more than 60 days apart. In the three instances where two cases had their onset less than 60 days apart in children from the same class, the illnesses occurred during summer holiday when the schools were not in session. The distribution of cases per class did not differ from the expectations of a Poisson distribution ($p=0.165$). To further verify the appropriateness of the randomization, the incidence of typhoid fever was also calculated as classes with typhoid cases per 100 classes vaccinated with each formulation of vaccine or with the control preparation. As shown in Table 1, this analysis provides virtually identical efficacy results as when cases per 10^5 schoolchildren are used in calculating efficacy.

Efficacy by year of surveillance is presented in Figure 1. The level of protection due to vaccine in enteric-coated capsules was particularly low in the third year of surveillance when it dropped to 10.9% from the levels of 34.0% and 48.7% vaccine efficacy recorded in years one and two of follow-up.

The efficacy of each formulation of Ty21a in relation to age is shown in Table 2. The degree of protection provided by the liquid formulation was similar in both the young (5-9 year old) (82.3% efficacy) and older ($\geq$ 10 years old) children (69.3% efficacy). In contrast, while the enteric-coated capsules conferred 51.1% protection in older children (p=0.06), there was no evidence of significant protection in the young (5-9 year old) children. The failure of enteric-coated vaccine to significantly protect children in the 5-9 year old age group was noted both in Sur Oriente (vaccine efficacy 16.5%) and in Norte (vaccine efficacy 18.4%). In older children (Table 2), the level of vaccine efficacy elicited by the liquid formulation (69.3%) was better than that stimulated by enteric-coated capsules (51.1%) but the difference was not significant (p=0.2).

DISCUSSION

The remarkable safety record of Ty21a live oral typhoid vaccine in more than 600.000, mostly pediatric, subjects who have received more than 1,400,000 doses of vaccine in prospective clinical trials (13) marks a notable improvement over the currently available, highly reactogenic, parenteral typhoid vaccine consisting of heat-phenolized *S. typhi* organisms (14,24-27).

Results of the 36 month field trial just completed in Santiago corroborate earlier observations that the formulation of Ty21a vaccine markedly influences the level of protection that can be achieved (9-14). Specifically, the data convincingly demonstrate a superiority of Ty21a administered in a liquid suspension over vaccine in enteric-coated capsules. The

difference between the formulations was most prominent in the younger schoolchildren (5-9 year olds) where the liquid formulation conferred 82.3% vaccine efficacy but the enteric-coated capsule formulation conferred only a disappointing 18.7% efficacy (p=0.000003).

To give perspective to the data from the Sur Oriente/Norte trial, they are compared with results from the two other randomized, placebo-controlled field trials in which enteric-coated capsules or a liquid suspension of Ty21a were administered to schoolchildren in the same dosage schedule as in the Sur Oriente/Norte trial. Age-specific analyses of protection conferred by a liquid suspension of Ty21a in Alexandria, Egypt (10) and of enteric-coated capsules in Area Occidente, Santiago (11) are summarized in Table 2, to be compared with the age-specific results of the current trial also shown in Table 2. In young children, the efficacy of the liquid formulation used in Sur Oriente/Norte (82.3%) (Table 2) approached the level of efficacy (96%) seen with the liquid formulation used in Alexandria (Table 2); furthermore, the 95% confidence intervals surrounding these two levels of vaccine efficacy are quite similar. Based on results of these two field trials, one can conclude that three doses (spaced 1-2 days apart) of Ty21a in a liquid suspension given to 5-9 year old children in endemic areas confer circa 82-96% protection against typhoid fever.

The level of protection stimulated by enteric-coated Ty21a in older children in Sur Oriente/Norte (51.1% efficacy) (Table 2) is lower than the 71.9% vaccine efficacy recorded in the same age group in the earlier Occidente trial (Table 2). However, the considerable overlap in the confidence intervals once again indicates that these different results are probably simply variations of the sort expected when independent field trials are carried out in distinct geographic sites or in the same site in different years. For example, in the WHO-sponsored field trials that evaluated killed whole cell parenteral typhoid vaccines, site-to-site variations of up to 15% were observed in the level of efficacy when the identical

vaccines were tested in different geographic areas (14,27-29). Thus, based on the data in Table 2, three doses of Ty21a in enteric-coated capsules offer circa 51-72% protection to older children in endemic areas.

The most notable difference observed in the current study versus the earlier Area Occidente trial is the poor efficacy of vaccine in enteric-coated capsules in 5-9 year olds: 16.8% versus 59.1% vaccine efficacy (Table 2). Even in this instance where the 95% confidence intervals surrounding the recorded efficacy (0-53% versus 16-80%) are more marked, they still overlap. Thus, the observed difference may also be simply due to chance. If this were true, comparing results from these two trials, one would estimate that in 5-9 year olds, three doses of Ty21a in enteric-coated capsules provide somewhere in the range of 17-51% protective efficacy against typhoid fever.

There are several possible explanations for the superiority of Ty21a when administered as a liquid suspension. Vaccine organisms may be hardier when reconstituted *in vitro*, before ingestion, than if they must recover from the lyophilized state immediately after release from a degraded capsule in the intestinal environment where they are exposed to bile acids, digestive enzymes and partially digested food. Moreover, a liquid suspension affords the vaccine organisms contact with the tonsils, an immunologic organ. This potentially important contact does not occur with vaccine in enteric-coated capsules.

A somewhat analogous comparison of the liquid and enteric-coated formulations of Ty21a was performed in Plaju, Indonesia, where preliminary results show the liquid formulation to be slightly but not significantly superior (30). Differences between the Indonesian trial and the Sur Oriente/Norte trial include an annual attack rate for typhoid fever in Plaju that was several-fold higner than in the Chilean trial and a different immunization schedule from that used in Chile (in Plaju three doses were given one week apart).

Results of the Sur Oriente/Norte field trial lead us to recommend the liquid formulation, especially for children. Not only was this formulation superior in efficacy at all ages but it was notably more practical for immunizing the youngest children (5-8 year olds), a proportion of whom had difficulty swallowing the enteric-coated capsules.

Besides the use of Ty21a to control disease in endemic areas (13,14), its role in immunizing travelers must be considered. One must exercise caution in extrapolating from results of a field trial of a typhoid vaccine in an endemic area to expectations of protection for travelers. Nevertheless, studies in volunteers carried out in the early 1970s (albeit with more and higher doses of Ty21a) show that Ty21a can protect North Americans against a relatively high inoculum of pathogenic *S. typhi* that caused experimental typhoid fever in 55% of unimmunized control volunteers (9). In the present field trial, the youngest children (5-9 year olds), who most closely resemble industrialized country travelers with respect to having the least background immunity from prior exposure to *S. typhi*, were impressively protected by the liquid formulation of Ty21a (81.9% efficacy). Together, these observations suggest that the liquid formulation of Ty21a should be preferred for immunizing travelers. Ty21a is licensed in many countries but is currently available only in enteric-coated capsules. Based on results of this field trial, efforts have been initiated to mass produce the liquid formulation.

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Table 1. Comparison of the efficacy of three doses of Ty21a live oral typhoid vaccine given in enteric-coated capsule or liquid formulations. 36 months of surveillance of a randomized, controlled, double-blind trial in Area Sur Oriente and Area Norte, Santiago, Chile

	Enteric		
	Liquid	Capsules	Placebo*
No. of children	36,623	34,696	10,302
Cases	23	63	28
Incidence/10^5	62.8[a]	181.6[b]	271.8[c]
Efficacy+	76.9%	33.2%	-
95% confidence interval	(60-87)	(0-57)	
No. of classes	2,369	2,367	687
Classes with typhoid	23	61	27
Classes with typhoid/100 classes	0.97[d]	2.57[e]	3.93[f]
Efficacy+	75.3%	34.6%	-
95% confidence interval	(56-85)	(0-59)	-

* The liquid and enteric-coated placebo (*Lactobacillus*) groups were combined as a single placebo group in calculating vaccine efficacy since the incidence of typhoid fever was so similar in the individual placebo groups: 14 cases among 5,450 recipients of liquid placebo (256.9 cases/10^5) and 14 cases among 4,852 recipients of enteric-coated capsule placebo (288.5 cases/10^5) ($p = 0.91$, Chi square with Yates correction).

\+ % vaccine efficacy was calculated by the formula:

$$\frac{\text{incidence in placebo group - incidence in vaccine group}}{\text{incidence in placebo group}} \times 100$$

a vs c, $p < 0.0000001$ (one tail); b vs c, $p = 0.048$ (one tail); a vs b, $p = 0.0000077$ (two tails); d vs f, $p < 0.0000001$ (one tail); e vs f, $p = 0.041$ (one tail); d vs e, $p = 0.00045$ (two tails). Statistical comparisons by Chi square with Yates correction.

Table 2. Analysis of efficacy by age of Ty21a live oral typhoid vaccine given as three doses in enteric-coated capsule or liquid formulations in a randomized, controlled, double-blind field trials in Area Sur Oriente and Area Norte, Santiago. Comparison with results of two earlier field trials in Alexandria, Egypt and Area Occidente, Santiago where three doses of Ty21a live oral typhoid vaccine in enteric-coated capsules or in a liquid formulation were given.

	Area Sur Oriente/Norte, Santiago			Alexandria, Egypt[-]		Area Occidente, Santiago [*]	
	Liquid	Enteric Capsules	Placebo[~]	Liquid	Placebo	Enteric Capsules	Placebo[-]
Age 5 - 9 years							
N	22,586	21,128	5,989	16,486	15,902	7,034	7,193
Cases	10	44	15	1	22	10	25
Incidence/10^5	44.3[a]	208.3[b]	250.5[c]	6.1[d]	138.3[e]	142.2[f]	347.6[g]
Efficacy	82.3%	16.9%	-	95.6		59.1	
95% confidence interval	(61-92)	(0-53)		(77-99)		(16-80)	
Age > 9 years							
N	14,037	13,568	4,313	-	-	15,134	14,711
Cases	13	19	13	-	-	13	45
Incidence/10^5	92.6[h]	140.0[i]	301.4[j]	-	-	85.9[k]	305.9[l]
Efficacy	69.3%	53.5%	-	-	-	71.9	-
95% confidence interval	(35-86)	(7-77)	-	-	-	(48-55)	-

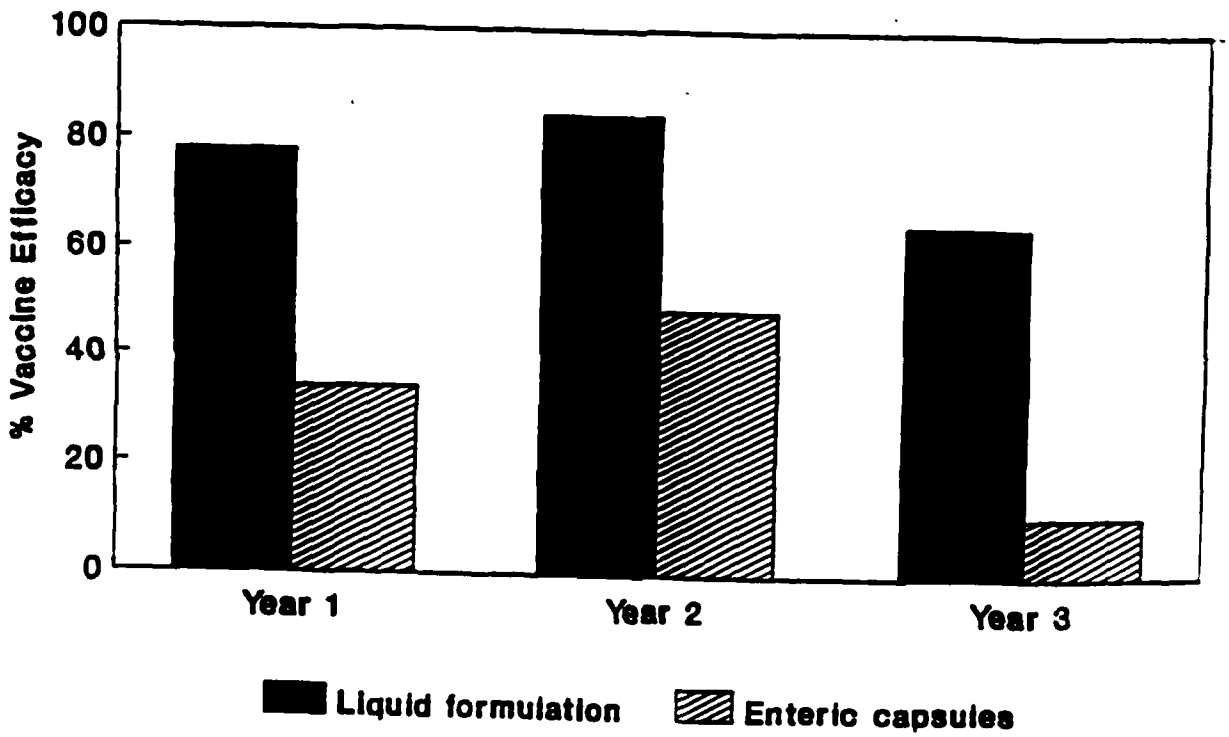
Note: Data from all three field trials represent results from 36 months of surveillance of schoolchildren who received three doses of vaccine or placebo within one week.

[-] Data from Wahdan et al (10). In this trial only 6 and 7 year olds were vaccinated.
[*] Data from Levine et al (11).
[~] Combined liquid and enteric-coated placebo groups.

a vs c, p = 0.0000026 (one tail); b vs c, p = NS; a vs b, p = 0.000002 (two tails)
d vs e, p = 0.000045 (two tails); f vs g, p = 0.021 (two tails)
h vs j, p = 0.0015 (one tail); i vs j, p = 0.024 (one tail); h vs i, p = NS
k vs l, p = 0.000029 (two tails)
All statistical comparisons by Chi square with Yates correction.

FIGURE LEGEND

Figure 1. The efficacy of Ty21a given to Chilean schoolchildren as either a liquid suspension of vaccine organisms or in enteric-coated capsules is shown for the first, second and third years of surveillance.



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